

An investigation into the α -adrenoceptor mediating renal nerve-induced calcium reabsorption by the rat kidney

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1 An investigation was undertaken in pentobarbitone-anaesthetized rats to determine the sub-type of α -adrenoceptor responsible for the renal nerve-induced increases in the reabsorption of calcium and sodium by the tubules of the kidney.

2 Stimulation of the renal nerves at low frequencies (0.8–1.5 Hz) did not change either renal blood flow or glomerular filtration rate but significantly reduced urine flow by 32%, calcium excretion by 36% and absolute and fractional sodium excretions by 36% and 22%, respectively.

3 In the presence of the selective α_1 -adrenoceptor antagonist prazosin, renal nerve stimulation (2–3 Hz) caused a significant reduction in renal blood flow of 7% but did not change either glomerular filtration rate, urine flow, calcium excretion or absolute and fractional sodium excretions.

4 During administration of the selective α_1 -adrenoceptor antagonist, idazoxan, renal nerve stimulation (1.0–1.5 Hz) significantly reduced renal blood flow by 4% and glomerular filtration rate by 7%; at the same time there were significant falls in urine flow of 43%, calcium excretion of 43% and absolute and fractional sodium excretions of 41% and 37%, respectively.

5 These results show that low frequency renal nerve stimulation causes an anticalciuresis, independent of renal haemodynamics, which represents an increase in tubular reabsorption of calcium. This effect was blocked by prazosin but not idazoxan which is consistent with the mediation of α_1 -adrenoceptors. The neurally-induced antinatriuresis also appeared to be dependent on the activation of α_1 -adrenoceptors.

Introduction

Evidence has accumulated to show that the renal sympathetic nerves exert an important control on many aspects of kidney function which include renal blood flow, renin release and excretion of sodium. The anatomical substrate for these effects are well founded, as the renal nerves have been shown to innervate both vascular smooth muscle (Barajas, 1978) as well as the epithelial cells of the tubules (Barajas *et al.*, 1984) of the rat kidney.

Recently it has emerged that low rates of renal nerve activation, due to either electrical or reflex stimulation which has no effect on either renal blood flow or glomerular filtration rate, can increase the rate of tubular sodium reabsorption (DiBona, 1982). It is apparent that the regulation of neurally induced tubular sodium reabsorption is mediated by α -adrenoceptors as the antinatriuresis of direct renal nerve

stimulation (Zambraski *et al.*, 1976) or reflex activation (DiBona & Johns, 1980) could be blocked with phenoxybenzamine or phentolamine. Further studies in which selective α -adrenoceptor agonists and antagonists were used have shown the adrenergic regulation of tubular sodium reabsorption to be of the α_1 -subtype (Hesse & Johns, 1985).

Although a great deal of attention has been focused on the neural regulation of tubular sodium reabsorption, there has been very little investigation of the influence of the renal nerves on the reabsorption of other ions. However, in a recent paper we showed that renal denervation was associated with a calciuresis and that renal nerve stimulation, at frequencies that had no effect on renal haemodynamics, resulted in a large anticalciuresis indicating that the renal nerves could have important effects on the reabsorption of calcium ions (Johns & Manitius, 1986).

The question therefore arises as to whether or not the neurally induced changes in calcium reabsorption

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are mediated by α -adrenoceptors and if so, which sub-type of α -adrenoceptor is involved. This was examined in the present study by electrically stimulating the renal nerves at low rates and determining their action on calcium reabsorption in the presence of either α_1 - or α_2 -adrenoceptor blockade.

Methods

Male Sprague-Dawley rats (360–380 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} intraperitoneally) and supplemental doses were administered every 15–20 min. The right carotid artery was cannulated for blood pressure measurements (a Statham P23ID pressure transducer connected to a Grass model 7D polygraph) and collection of blood samples. The left jugular vein was cannulated to allow infusion of saline (NaCl 150 mM) which was given at 6.0 ml h^{-1} for the duration of the experiment.

An abdominal mid-line incision was used to expose the left kidney, the left renal artery was cleared and an electromagnetic flowmeter probe (Carolina EP100 series) fitted for direct renal blood flow measurement (Carolina FM510 flowmeter linked to a Grass model 7D polygraph). Urine was collected from a cannula placed in the left ureter. All nerves running to the left kidney from the coeliac and aortic ganglia were identified, by use of a Zeiss model 212 surgical microscope, dissected clear for a short length such that they could be placed on bipolar silver wire electrodes, then sectioned. Their functionality was established by showing that a 5–10 s period of 10 Hz stimulation (15 V, 0.2 ms) caused a blanching of the kidney.

Renal function measurements

At the end of surgery, 2 ml of saline containing inulin (10 mg ml^{-1}) was given intravenously over 2 min and the saline infusion changed to one containing inulin, 10 mg ml^{-1} , which was infused for the remainder of the experiment. A 2 h period was allowed for recovery from surgery before measurements were started.

Each experiment consisted of five consecutive 20 min clearance periods, two before and two following a period during which the renal nerves were electrically stimulated. No urine sample was collected during the first 5 min of renal nerve stimulation or for the 5 min immediately after cessation of stimulation in order to allow pre-formed urine to clear from the cannula.

Arterial blood samples (0.35 ml) were taken at the beginning and end of the first and second pair of clearance periods, immediately centrifuged, the plasma removed for storage in the deep freeze and the red cells were resuspended in an equal volume of saline and returned to the animal as quickly as possible.

Average blood pressure and renal blood flow were measured over each clearance period by use of a BBC microcomputer and Unilab interface linked to a Torch Z80 disc drive which was programmed to accept input data from the Grass polygraph (Emmerson & Johns, 1986).

Plasma and urinary concentrations of inulin were estimated as previously described (Johns *et al.*, 1976) and glomerular filtration rate was calculated as the clearance of inulin (Arundell & Johns, 1982). Inulin was estimated in plasma and urine following deproteinisation (Somogyi, 1930), according to the method of Bojesen (1952). Urinary and plasma levels of sodium were measured with a Beckman flame photometer and urinary calcium concentrations were estimated with a Perkin-Elmer 2380 atomic absorption spectrophotometer.

Experimental protocols

Three groups of animals were studied, those in which saline was infused throughout the experiment, a second in which prazosin was given and a third in which idazoxan was administered.

Saline infusion: these animals acted as controls to assess the changes induced by renal nerve stimulation at frequencies between 0.8–1.5 Hz which were below the threshold to cause changes in renal blood flow.

Prazosin infusion: 1.5 h after completion of surgery, prazosin was given i.v. as a bolus of $250 \mu\text{g kg}^{-1}$, over 1 min, which was followed by a constant i.v. infusion of $50 \mu\text{g kg}^{-1} \text{ h}^{-1}$ contained in the saline infusion. The vasopressor and renal vasoconstrictor effects of phenylephrine ($2 \mu\text{g}$) and the vasopressor effects of UK 14304 (5-bromo-6[2-imadiazolin-2-ylamino]-quinoxaline) ($2 \mu\text{g}$) were obtained 10 min before the start of drug infusion. Fifteen minutes after the start of prazosin infusion the responses to phenylephrine but not UK 14304, were found to be abolished.

Idazoxan infusion: 1.5 h following completion of surgery the α_2 -adrenoceptor antagonist, idazoxan (RX781094) was given as an i.v. bolus of 1 mg kg^{-1} over 1 min and was followed by a sustaining i.v. infusion of $250 \mu\text{g kg}^{-1} \text{ min}^{-1}$ in the saline infusion. The vasopressor and renal vasoconstrictor actions of phenylephrine ($2 \mu\text{g}$) and vasopressor effects of UK 14304 ($2 \mu\text{g}$) were obtained 10 min before the administration of idazoxan. Fifteen minutes after the start of the idazoxan the responses to UK 14304, but not phenylephrine, were abolished.

Statistics

The absolute and percentage changes quoted in the

text represent a mean of the individual changes recorded in each animal. The renal responses to nerve stimulation were measured by taking the average value of the two clearances before and two after stimulation and comparing it with the value obtained during nerve stimulation. This was done in order to take into account any spontaneous changes which could occur during the course of the experiment. There were no statistical differences between pre- and post-stimulation values. All data are expressed as means \pm s.e.mean. The paired Student's *t* test was used for intragroup analysis and the unpaired Student's *t* test for intergroup analysis. Differences between means were considered significant at the 5% level.

Results

The blood pressure and renal responses to low frequency renal nerve stimulation in animals infused with saline are presented in Table 1. Stimulation of the renal nerves had no effect on blood pressure and was not sufficient to change either renal blood flow or glomerular filtration rate. However, there were significant reductions in urine flow of 32% ($P < 0.01$), calcium excretion of 36% ($P < 0.01$) and absolute and fractional sodium excretions of 36% ($P < 0.01$) and 22% ($P < 0.01$), respectively, during the period of nerve stimulation. All variables returned to pre-stimulation values during the recovery period.

In the group of animals given prazosin, 2 μ g phenylephrine raised blood pressure by 33 ± 4 mmHg and reduced renal blood flow by 7.7 ± 0.9 ml min⁻¹ kg⁻¹ while 2 μ g UK 14304 increased blood pressure by 25 ± 3 mmHg and reduced renal blood flow by 1.2 ± 0.1 ml min⁻¹ kg⁻¹. Fifteen minutes after the start of the prazosin infusion, the same dose of phenylephrine decreased blood pressure by 2 ± 2 mmHg and reduced renal blood flow by 0.1 ± 0.1 ml min⁻¹ kg⁻¹ while the same dose of

UK 14304 increased blood pressure by 20 ± 3 mmHg and reduced renal blood flow by 1.2 ± 0.1 ml min⁻¹ kg⁻¹. Table 2 presents the blood pressure and renal responses to renal nerve stimulation during the administration of the selective α_1 -adrenoceptor antagonist, prazosin. Basal blood pressure in this group of animals was significantly ($P < 0.01$) lower than that observed in the animals given only saline (Table 1) but it remained stable throughout the experimental period. All renal variables had values similar to those observed in animals infused with saline (Table 1). During the period of renal nerve stimulation there was a significant ($P < 0.05$) reduction in renal flow of approximately 7%, while glomerular filtration rate was not changed. There were no significant alterations in either urine flow, calcium excretion, absolute or fractional sodium excretions when the nerves were stimulated which was a pattern significantly (in all cases $P < 0.05$) different from the responses of these variables observed in the saline infused group of animals (Table 1). A comparison of these excretory changes are shown in Figure 1.

In the animals treated with idazoxan, prior to the antagonist, 2 μ g phenylephrine raised blood pressure by 28 ± 3 mmHg and decreased renal blood flow by 6.8 ± 0.5 ml min⁻¹ kg⁻¹ and 2 μ g UK 14304 increased blood pressure by 15 ± 3 mmHg and decreased renal blood flow by 1.0 ± 0.1 ml min⁻¹ kg⁻¹. Fifteen minutes after the start of the idazoxan infusion, administration of the same dose of phenylephrine increased blood pressure by 29 ± 3 mmHg and decreased renal blood flow by 6.8 ± 0.5 ml min⁻¹ kg⁻¹ whereas the same dose of UK 14304 increased blood pressure by 2 ± 1 mmHg and reduced renal blood flow by 0.1 ± 0.01 ml min⁻¹ kg⁻¹. Table 3 shows the blood pressure and renal haemodynamic responses to renal nerve stimulation in the presence of the selective α_2 -adrenoceptor antagonist, idazoxan. The blood pressure in this group of animals was significantly ($P < 0.02$) less than that observed in the saline-infused

Table 1 Effect of low-frequency renal nerve stimulation on blood pressure and renal function ($n = 7$)

	Control	Experimental	Recovery
Blood pressure (mmHg)	134 \pm 4	131 \pm 3	138 \pm 5
Renal blood flow (ml min ⁻¹ kg ⁻¹)	14.1 \pm 1.0	14.3 \pm 1.1	13.9 \pm 1.2
Glomerular filtration rate (ml min ⁻¹ kg ⁻¹)	3.45 \pm 0.37	3.41 \pm 0.58	3.89 \pm 1.03
Urine flow (μ l min ⁻¹ kg ⁻¹)	49.8 \pm 7.8	35.7 \pm 6.5***	56.0 \pm 10.8
Absolute calcium excretion (nmol min ⁻¹ kg ⁻¹)	103.6 \pm 17.8	74.3 \pm 15.8**	124.6 \pm 27.1
Absolute sodium excretion (μ mol min ⁻¹ kg ⁻¹)	11.6 \pm 1.9	8.0 \pm 1.9***	12.5 \pm 2.7
Fractional sodium excretion (%)	2.27 \pm 0.45	1.77 \pm 0.57***	2.93 \pm 1.04

The *P* values represent a comparison between the mean of the control and recovery values with that obtained during the renal nerve stimulation period (experimental): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Frequencies of renal nerve stimulation ranged from 0.8 to 1.5 Hz.

Table 2 Effect of low-frequency renal nerve stimulation on blood pressure and renal function in the presence of prazosin ($n = 8$)

	Control	Experimental	Recovery
Blood pressure (mmHg)	110 \pm 4	114 \pm 4	111 \pm 4
Renal blood flow (ml min ⁻¹ kg ⁻¹)	15.5 \pm 1.7	14.3 \pm 1.5*	15.5 \pm 2.1
Glomerular filtration rate (ml min ⁻¹ kg ⁻¹)	3.87 \pm 0.41	3.57 \pm 0.34	3.34 \pm 0.21
Urine flow (μ l min ⁻¹ kg ⁻¹)	52.3 \pm 9.1	47.7 \pm 5.8	50.9 \pm 6.4
Absolute calcium excretion (nmol min ⁻¹ kg ⁻¹)	65.7 \pm 15.8	73.9 \pm 12.6	74.9 \pm 15.8
Absolute sodium excretion (μ mol min ⁻¹ kg ⁻¹)	11.5 \pm 2.1	11.0 \pm 1.9	12.7 \pm 2.2
Fractional sodium excretion (%)	2.24 \pm 0.50	2.27 \pm 0.46	2.67 \pm 0.48

The P values represent a comparison of the mean of the control and recovery values with that obtained during the renal nerve stimulation period (experimental): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Prazosin was given as an i.v. bolus of 250 μ g kg⁻¹ followed by a continuous i.v. infusion of 50 μ g kg⁻¹ h⁻¹. The frequencies of renal nerve stimulation used ranged between 2–3 Hz.

animals but was similar to that of the prazosin group. Blood pressure in the animals given idazoxan remained at a steady level over the time course of the experiment. Stimulation of the renal nerves in the idazoxan-treated animals caused small, but significant, reductions, of approximately 4% ($P < 0.05$) in renal blood flow, and of some 7% ($P < 0.01$) in glomerular filtration rate, although the magnitude of these responses was not distinguishable from those observed in the two other groups of animals. There were significant reductions in urine flow of 43% ($P < 0.001$), calcium excretion of 43% ($P < 0.001$),

absolute sodium excretion of 41% ($P < 0.001$) and fractional sodium excretion of 37% ($P < 0.001$) during the period of renal nerve stimulation and all these variables returned to values not significantly different from control values during the recovery period. The magnitudes of these excretory responses to nerve stimulation were similar to those obtained in the animals infused with saline, but were significantly ($P < 0.001$, $P < 0.01$, $P < 0.001$, $P < 0.01$, respectively) larger than those observed in the animals given prazosin. Figure 1 presents comparisons of the percentage changes of these excretory responses.

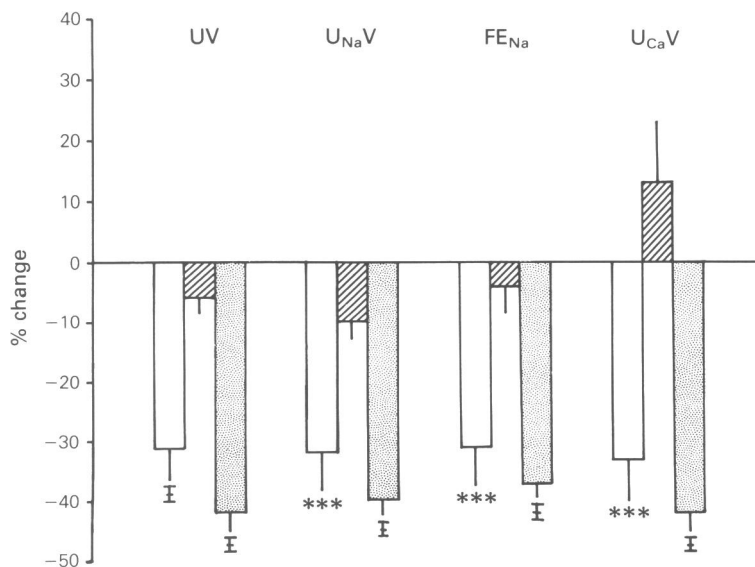


Figure 1 Percentage changes which take place during low frequency renal nerve stimulation in urine flow (UV), calcium excretion (U_{Ca}V), absolute sodium excretion (U_{Na}V) and fractional sodium excretion (FE_{Na}). Open columns, control ($n = 7$); hatched columns, prazosin ($n = 8$); stippled columns, idazoxan ($n = 7$). *** = $P < 0.001$; † = $P < 0.001$.

Table 3 Effect of low-frequency renal nerve stimulation on blood pressure and renal function in the presence of idazoxan ($n = 7$)

	Control	Experimental	Recovery
Blood pressure (mmHg)	117 \pm 4	120 \pm 4	121 \pm 4
Renal blood flow (ml min ⁻¹ kg ⁻¹)	14.2 \pm 0.8	13.2 \pm 0.7*	13.5 \pm 0.8
Glomerular filtration rate (ml min ⁻¹ kg ⁻¹)	3.48 \pm 0.25	3.04 \pm 0.28**	3.03 \pm 0.30
Urine flow (μ l min ⁻¹ kg ⁻¹)	49.5 \pm 5.6	29.9 \pm 2.7***	56.3 \pm 6.4
Absolute calcium excretion (nmol min ⁻¹ kg ⁻¹)	81.7 \pm 16.3	50.4 \pm 10.1***	95.2 \pm 20.1
Absolute sodium excretion (μ mol min ⁻¹ kg ⁻¹)	10.8 \pm 1.3	6.9 \pm 0.7***	12.6 \pm 1.3
Fractional sodium excretion (%)	2.07 \pm 0.35	1.51 \pm 0.21***	2.77 \pm 0.40

The P values represent a comparison of the mean of the control and recovery values with that obtained during the period of renal nerve stimulation (experimental): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Idazoxan was given as an i.v. bolus dose of 1 mg kg⁻¹ followed by a continuous i.v. infusion of 250 μ g kg⁻¹ h⁻¹. Frequencies of nerve stimulation ranged from 1.0 to 1.5 Hz.

Discussion

The aim of this study was to determine whether the decrease in calcium excretion caused by renal nerve stimulation, at low frequencies which did not affect renal haemodynamics, was mediated by α_1 - or α_2 -adrenoceptors. This was done by using the selective α_1 -adrenoceptor antagonist, prazosin (Cambridge *et al.*, 1977) and the selective α_2 -adrenoceptor antagonist, idazoxan (Doxey *et al.*, 1983) which are among the most selective of the α -adrenoceptor antagonists available at present.

An important objective was to stimulate the renal nerves at frequencies which caused minimal or no changes in renal blood flow and in the group of animals given only saline this was achieved. At the same time there were large reductions in the output of water and calcium as well as sodium. This reduction in calcium excretion has been taken to be a consequence of a direct action of the nerves on the renal tubules, probably as a result of the release of noradrenaline from those nerve terminals ending in close proximity to the epithelial cells (Barajas *et al.*, 1984). At this site it is likely that the calcium transporting processes of the tubular cells are stimulated which produces a reduction in the excretion of this ion. A similar fall in the excretion of sodium was also observed in the present study and is a finding which has been reported consistently during renal nerve stimulation at low rates (DiBona, 1982). It is generally accepted that this action of the renal nerves is a direct stimulatory effect on the movement of sodium ions across epithelial cells which occurs primarily at the proximal tubule (Bello-Reuss *et al.*, 1976; Pelayo *et al.*, 1983) and the ascending limb of the loop of Henlé (DiBona & Sawin, 1982; Bencsath *et al.*, 1985). Because of the parallel changes in the reabsorption of calcium and sodium,

the observations of the present study would suggest that the stimulation of calcium reabsorption by the renal nerves occurs at similar sites along the nephron and it is known that up to 60% of non-hormonally regulated calcium reabsorption takes place along the proximal tubule and the loop of Henlé (Dennis *et al.*, 1979). This relationship is by no means proven by the present studies; however, it is recognised that the major reabsorption pathway for calcium ions at these sections of the nephron is via the sodium/potassium ATPase-driven sodium/calcium counter transporter system at the basolateral membranes of the tubular cells (Katz, 1986).

Administration of prazosin clearly caused a selective inhibition of the α_1 -adrenoceptor-mediated vasopressor and renal vasoconstrictor responses to phenylephrine, although the basal levels of renal haemodynamics and fluid excretion were similar to those of the animals infused with saline. In spite of the significant reduction in renal blood flow during renal nerve stimulation in the presence of prazosin, the reabsorption of calcium did not change and such an inhibition of the response would indicate that α_1 -adrenoceptors were involved in renal nerve activation of the calcium reabsorptive processes. In an attempt to establish more firmly this contention, a further group of animals undergoing α_2 -adrenoceptor blockade were studied. The data showed that administration of idazoxan caused a selective blockade of the α_2 -adrenoceptor-mediated vasopressor and renal vasoconstrictor responses to UK 14304. In these animals stimulation of the renal nerves was associated with a large reduction in calcium excretion, similar in magnitude to that obtained in the absence of drug, and provided no evidence of a role for postsynaptic α_2 -adrenoceptors in the nerve-induced stimulation of calcium reabsorption. Together, these findings support the observa-

tions with prazosin and strengthen the conclusion that α_1 -adrenoceptors mediated the adrenergic influence on the calcium reabsorptive processes of the tubular cells.

The results of the present study also indicated that α_1 -adrenoceptors were necessary for the activation of tubular sodium reabsorption as renal nerve stimulation in the presence of prazosin did not change sodium output whereas during administration of idazoxan the magnitude of the increases in tubular sodium reabsorption were similar to those in the animals receiving only saline. A role for α_1 -adrenoceptors in the adrenergic stimulation of tubular sodium reabsorption in the rat was recently reported in *in vitro* studies by Smyth *et al.* (1985) and the present study using *in vivo* experiments support these *in vitro* findings. It is becoming apparent that this particular end-point of adrenergic activity within the kidney also requires α_1 -adrenoceptors in the rabbit (Hesse & Johns, 1984a; 1985) and the dog (Osborn *et al.*, 1983).

During infusion of both prazosin and idazoxan there were reductions in renal blood flow and glomerular filtration rate when the renal nerves were stimulated and it is possible that these haemodynamic changes could have contributed to the calcium and sodium retention observed. However, in a previous study in the rabbit (Hesse & Johns, 1984b) it was found that nerve-induced reductions in renal blood flow by up to 15% did not significantly change the magnitude of the antinatriuresis from that observed when the nerves were stimulated at rates which had no renal haemodynamic effect. Further, in the present study the significant reductions in renal blood flow and falls in glomerular filtration rate during renal

nerve stimulation in the presence of prazosin and idazoxan were not statistically different yet the magnitude of the changes in calcium and sodium were very different. Therefore it is unlikely that these small changes in renal haemodynamics contributed meaningfully to the renal nerve-induced changes in the tubular handling of calcium and sodium.

The results of the present study in the rat show that low frequency renal nerve stimulation, which had minimal haemodynamic influences, caused large reductions in the excretion of calcium and this has been taken to reflect an increase in the tubular transport of this ion probably at the proximal tubule and ascending limb of the loop of Henlé. The studies using the α_1 - and α_2 -adrenoceptor antagonists, prazosin and idazoxan, demonstrated that the renal nerves exerted their action on calcium reabsorption by means of α_1 -adrenoceptors. The present observations also showed that the renal nerve-induced increases in tubular sodium reabsorption were also dependent on the activation of α_1 -adrenoceptors. There is evidence that the reabsorption of calcium at the proximal tubule and the loop of Henlé is parallel to and may be dependent on the active reabsorption of sodium (Ullrich *et al.*, 1976) but whether the renal nerves exert their α_1 -adrenoceptor-mediated effects directly on calcium reabsorption or indirectly, via stimulation of sodium transport across the epithelial cells, remains to be clarified.

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